

## **Imbalance of certain components of saliva in patients with fluorosis**

Ludmila Gavriiliuc, Elena Stepco, Pavel Godoroja, Vladimir Hornet  
Chisinau, Republic of Moldova

### **Summary**

**Fluorosis, caused by long-term intake of high levels of fluoride, is characterized by clinical manifestations in bones and teeth. However, detrimental effects of high-fluoride intake are observed in soft tissues also. Although fluorosis is irreversible, it could be prevented by appropriate and timely intervention through understanding the process at biochemical and molecular levels. Increased production of reactive oxygen species (ROS) and lipid peroxidation has been considered to play an important role in the pathogenesis of chronic fluoride toxicity.**

**Saliva is a biological liquid of the human organism, and may be a reflection of the metabolic state. The concentration of calcium ( $\text{Ca}^{2+}$ ), proline, hydroxyproline, creatinine and activity of alkaline phosphatase were determined in the saliva of patients with fluorosis. An imbalance has been determined in salivary components of patients with fluorosis. Correlation analysis among some biochemical indexes in the patients with fluorosis indicated metabolic imbalance.**

**Key words: fluorosis, saliva, creatinine, calcium, alkaline phosphatase.**

### **Introduction**

Fluorine is the most widespread active halogen. Fluorine and fluorides are used in industry, agriculture, medicine and dentistry. For example, fluoracetate is used as poison for rodents. Fluoruracil is used as an anticancer drug, an irreversible inhibitor of thymidilate syntetase and TMP synthesis and is needed for DNA production. Sodium fluoride can induce DNA damage and apoptosis in rats' brain [1, 2]. Fluoride can inhibit the synthesis of type I collagen [3].

Chronic oxidative stress entails numerous pathological consequences. Fluoride accumulation was observed in the brain of rats exposed to chronic high-fluoride intake through drinking water [4]. Intake of high levels of fluoride is known to cause structural changes [5, 6], altered activities of enzymes [7], and metabolic lesions [2] in the brain of experimental animals.

Increased free radical generation and lipid peroxidation (POL) are proposed to mediate the

toxic effects of fluoride on soft tissues and organs (liver, kidneys, brain, etc.) and skeletal, teeth. Fluorosis, caused by long-term intake of high levels of fluoride, is characterized by clinical manifestations in bones and teeth [8, 9]. Fluorosis is a serious public health problem in many parts of the world, where drinking water contains more than 1 ppm of fluoride (India, Canada, China, USA, Romania, etc.) [10].

In the Republic of Moldova we have a rather poor ecological situation: high level of pesticides, heavy metals, etc. In certain villages/regions fluoride has turned the ground water into slow poison. Many villagers are intoxicated with fluoride (Calaras, Ungeni, Anenii Novi, Chadir-Lunga, Hincesti, etc.).

Treatment of fluorosis, which affects both young and old alike, has posed a daunting task to the medical fraternity. Children have been affected by fluorosis of the teeth, that is one of the clinical manifestations of disease and metabolic disturbance.

Saliva is a biological liquid of human organism and may be a reflection of the metabolic state. Salivary indexes (parameters) can be clinico-diagnostical means [11, 12].

The **purpose** of this investigation is the comparative examination of salivary parameters in adult patients and children with fluorosis.

## Materials and Methods

Fifty-three patients divided into the following groups were examined: 1 - healthy children (12 in total, control group); 2 - healthy adult patients (15 in total, control group); 3 - children with fluorosis (11 in total, 5-14 years old); 4 - adult patients with fluorosis (15 in total, 18-37 years old). Patients with fluorosis had the first or second stage of the disease (mild or severe chronic fluorosis). Saliva was collected in the morning, before breakfast and centrifuged at 600 g during 10 min. Centrifuged of saliva were used for examination. When using SP "Humalyzer 2000" (Germany) the following parameters were determined in saliva: content of creatinine [13], proline and hydroxyproline [14], calcium-ions ( $\text{Ca}^{2+}$ ) [15], and the activity of alkaline phosphatase [16]. Salivary protein was determined by Watanabe N. method [17]. We used the "Human" GmbH and "DiaSys International" (Germany) reagents for salivary indexes determination. All solutions for examination were prepared by using deionized water.

The results were calculated with the help of statistical Student method [18]. For the examination of interrelations between salivary parameters Spirmen method of nonparametric correlation was used [18].

## Results and Discussion

Daily salivary composition and secretion are variable. Proceeding from this we calculated concentrations of salivary parameters according to the protein content in saliva. Our results after examining creatinine, proline, hydroxyproline, calcium in the saliva of healthy and fluorosis patients are shown in *Table 1*.

Our results evidenced that, the parameters in the saliva of patients with fluorosis differed from the parameters of control (healthy) patients group. The creatinine content per liter of saliva

in children-patients with fluorosis decreased slightly (83.3%), and according to g of protein content, this difference was statistical (38.1%;  $P < 0.05$ ). The protein concentration in the saliva of adult patients with fluorosis was similar to the protein concentration in the saliva of the control group. In children with fluorosis the protein concentration in saliva was higher (251.9%) than in healthy children. Determination of creatinine concentration in the saliva of adult patients with fluorosis displayed a decrease of its content both per liter of saliva (61.3%), and per g of protein (36.3%).

Creatinine and urea production are interrelative processes. Creatine is used for creatinine synthesis, and is produced from the amino acid arginine, an intermediate of urea production. One of the intermediates of creatine synthesis is ornithine, a substrate for the first chemical reaction of urea production (ornithine cycle). Decreasing creatine content leads, accordingly, to decreased creatinine production. In our previous investigation, urea concentration in the saliva of patients with fluorosis was decreased in comparison with the control group [19].

Collagen is a widespread protein of the human organism [20]. It makes one third of all proteins of human organism. One of the characteristics of collagen is a high content of amino acids, proline and 4-hydroxyproline, in its primary structure. From this position the determination of proline and hydroxyproline in the saliva of patients with fluorosis may be of great interest. Collagen is a predecessor for production of bone and teeth.

Determination of proline and hydroxyproline content (*Table 1*) in saliva of adult patients with fluorosis was 336.3% ( $P < 0.01$ ) per liter of saliva in comparison with the healthy group, but there was no difference when based to g of protein (115.6%;  $P > 0.05$ ). In children patients with fluorosis, the concentration of proline and hydroxyproline in saliva decreased (87.6%), and according to g of protein, it was 45.3% ( $P < 0.01$ ).

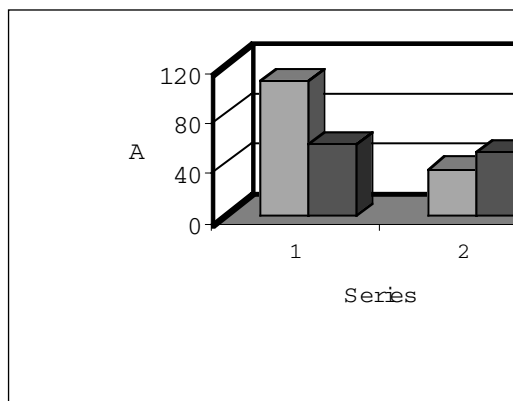
Fluoride can inhibit the synthesis of type I collagen [3]; also fluoride influences the expression level of type I collagen of rats osteoblasts. *Miao Q. et al.* [3] concluded that inhibition of collagen synthesis might be one of the factors leading to development of skeletal fluorosis. Our results may be the confirmation of collagen synthesis disturbance in patients with fluorosis.

**Table 1.** The content of creatinine, urea, proline, hydroxyproline, thiocyanate ions, chloride ions in the saliva of patients with periodontitis during treatment

Parameters		Healthy children	Healthy adults	Children with fluorosis	Adults with fluorosis
		(12)	(15)	(11)	(15)
Creatinine	a)	0.218	0.531	0.182	0.325*
	b)	0.353	0.704	0.136*	0.257*
Proline and hydroxyproline	a)	47.39	18.37	41.52	61.68*
	b)	66.93	36.24	30.35*	41.83
Calcium	a)	1.783	2.202	1.148*	1.921*
	b)	2.976	3.373	0.625*	1.616*

Content of creatinine (mmol), proline and hydroxyproline (mmol), calcium (mmol) in saliva: a) per liter (l); b) based to g of protein. Symbol \* - statistical difference ( $P < 0.05$ )

Moreover, the described parameters of saliva in the patients (creatinine, proline, hydroxyproline, protein) reflected the protein metabolism; the important clinico-diagnostical value for this pathology may be the examination of alkaline phosphatase activity. The results of the examination of alkaline phosphatase activity in the patients' saliva are presented in *Figure 1*. In children patients with fluorosis, the activity of alkaline phosphatase in saliva based to g of protein (specific activity) was 52.8% ( $P < 0.01$ ). On the contrary, in adult patients with fluorosis, the activity of alkaline phosphatase in saliva increased (127.1%;  $P < 0.05$ ) per liter of saliva and the specific activity (137.8%;  $P < 0.02$ ) also.

**Figure 1.** Activity of alkaline phosphatase in the patients with fluorosis

A - activity of alkaline phosphatase (IU/g of protein).  
1 - children (left - healthy; right - patients with fluorosis).  
2 - adult patients (left - healthy subjects; right - patients with fluorosis)

The alkaline phosphatase enzyme exists in all tissues of the human organism. A high level

of enzyme is present especially in bones, liver, and kidneys. Examination of alkaline phosphatase activity in children with fluorosis is of special value, because the activity of this enzyme may be changed before the clinical manifestations of the disease and decrease of inorganic phosphate concentration. Such phenomenon takes place, for example, in children with rickets. Decreased activity of alkaline phosphatase in children contributes to a decrease of the osteoblastic processes [21]. Our results are in accordance with this statement. Besides that, our data concerning the low level activity of alkaline phosphatase in the saliva of patients with fluorosis are correlated with the content of inorganic phosphate in patients' saliva [22]. The low level of alkaline phosphatase activity in blood and urine of children patients (8-15 years old) with fluorosis was determined by *Gao Y. et al.* [23]. The experimental results about  $Mg^{2+}$  ions as activators and  $F^{-}$  ions as inhibitors of alkaline phosphatase are well known [24]. Increased alkaline phosphatase activity in adult patients with fluorosis may be the result of chronic intoxication by  $F^{-}$  ions, conducting to the disturbance of liver functions and cholestasis [25].

Mineral components of saliva constitute one third of all salivary substances, which play an important role for teeth, soft tissues and mouth enzymes. Calcium ( $Ca^{2+}$ ) is one of main elements necessary for bones and teeth formation. It is also needed for many processes of the human organism: moving of nerve impulse, contraction and tonus of muscles, blood clotting (the IV<sup>th</sup> plasmic factor), stabilizer for alfa-amylase, etc. The determination of  $Ca^{2+}$  ions concentra-

tion in the saliva of patients with fluorosis was 87.2% per liter of saliva in adult patients ( $P < 0.05$ ), and in children - 64.4% ( $P < 0.01$ ). Data according to g of protein were significantly lower than in healthy groups: adult patients (47.9%;  $P < 0.01$ ) and children patients (20.9%;  $P < 0.01$ ). It is logical to assume that all the above-mentioned processes with  $\text{Ca}^{2+}$  ions participation may be changed in fluorosis as well.

Another approach was the correlation analysis between the salivary parameters of patients with fluorosis. The correlation analysis indicated a significant relationship between alkaline phosphatase activity and content of imino acids (proline and hydroxyproline) in the saliva of healthy adult patients ( $r = 0.721$ ;  $P < 0.01$ ), and healthy children patients ( $r = 0.945$ ;  $P < 0.001$ ). Alkaline phosphatase activity was not correlated with imino acids concentrations in the adult patients with fluorosis ( $r = 0.172$ ;  $P > 0.05$ ), but in children patients with fluorosis there was strong interrelation ( $r = 0.911$ ;  $P < 0.001$ ).

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## Conclusion

Due to our results, we can confirm that in patients with fluorosis an imbalance between salivary parameters takes place, as the result of chronic intoxication with fluoride contained in drinking water. Our investigation showed a similar picture of metabolic processes imbalance both in adult patients and children patients with fluorosis. At the same time, certain differences were noted between the metabolic disturbances of the salivary parameters in adult patients and children patients with fluorosis.

In all fluoride-endemic regions of Moldova it is necessary to carry out prophylactic actions, especially laying emphasis on small schools, pregnant women and feeding mothers. This prophylaxy action will decrease the risk of chronic intoxication by fluorides and will correct the metabolic imbalance in patients with fluorosis.

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Correspondence to: Professor Ludmila Gavriiuc - M.D., Biochemistry and Clinical Biochemistry Department, "Nicolae Testemitanu" State University of Medicine and Pharmacy. Bdul. Stefan cel Mare, no. 165, Chisinau, Republic of Moldova. E-mail: gavrlu@mail.md